# Understanding Xylose Metabolism of *Scheffersomyces stipitis* Through a Central Carbon Metabolic Network Model

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***Abstract***

The conversion of pentose into ethanol is one of the major barriers of industrializing lignocellulosic ethanol processes. As one of the most promising native strains for pentose fermentation, *Scheffersomyces stipitis* (formerly known as *Pichia stipitis*) has been widely studied for its xylose fermentation. In spite of the abundant experimental evidence regarding ethanol and by-products production under various aeration conditions, the mathematical descriptions of the processes are rare. In this work, the constraint-based metabolic network model for the central carbon metabolism of *S. stipitis* was reconstructed by integrating genomic (P. stipitis v2.0, KEGG), biochemical (ChEBI, PubChem) and physiological information available for this microorganisms and other related yeast. The model consists of the stoichiometry of metabolic reactions, the biosynthetic requirements for growth and other constraints. Flux Balance Analysis is applied to characterize the phenotypic behavior of *S. stipitis* grown on xylose. The model predictions are in good agreement with published experimental results. In addition, a series of specially designed *in silico* experiments was performed, and PCA has been applied to analyze the results to elucidate the redox balance of *S. stipitis* for xylose fermentation. The analysis revealed key metabolic reactions related to redox homeostasis and could provide important insights into cofactor engineering of xylose metabolism.

***Keywords***

*Scheffersomyces stipitis; Xylose Metabolism; Redox Balance; PCA; Cofactor Engineering; Systems Biology*

## Introduction

Lignocellulosic ethanol, considered as the second-generation biofuel (Naik, S.N., et al., 2010), has received increasing attention in recent years due to increased demand for fuel and expected depletion of fossil fuels (Eisentraut, A., 2010). Efficient utilization of xylose is one of the biggest obstacles for commercial production of lignocellulosic ethanol (Margeot, A., et al., 2009). *Scheffersomyces stipitis* (formerly known as *Pichia stipitis*) (Kurtzman, C.P. and M. Suzuki, 2010) has a set of physiological traits that make it potentially a valuable candidate for lignocellulosic ethanol production (Jeffries, T.W. and J.R.H. Van Vleet, 2009). It is considered as one of the few yeasts that can metabolize xylose in high efficiency with few by-products (Jeffries, T.W., and Y.S. Jin, 2000). It has been shown that oxygen availability plays a critical role in xylose metabolism of *S. stipitis* due to redox balance (Jeffries, T.W. and J.R.H. Van Vleet, 2009). Therefore, tuning up the redox conditions has significant impact on ethanol production of *S. stipitis*, particularly for xylose fermentation. However, in spite of the abundant experimental evidence, little is known about the cellular details on how redox balance affects xylose metabolism.

Constraint-based stoichiometric metabolic network modeling has been proven to be an effective way to study the metabolism of many microorganisms. Among the many approaches, Flux Balance Analysis (FBA) has been adapted in many research and can be used to study many aspects of biochemical networks (Orth, J.D., et al., 2010). However, one of the difficulties in existing metabolic network modeling is how to identify key reactions among hundreds or thousands reactions when cellular metabolism is influenced by a given factor. To address this challenge, in this work, we developed the central carbon metabolic model for *S. stipitis*, and proposed a new approach to analyze xylose metabolism by integrating Flux Balance Analysis (FBA) and Principal Component Analysis (PCA). The combination of FBA and PCA revealed that the key metabolism details of how different oxygen supply rates would cause metabolism shifts. The

influences of cofactor specificity of critical enzyme in xylose metabolism, xylose reductase (XR), was also studied. The PCA analysis results provide valuable insights into possible cofactor engineering of *S. stipitis*.

## Methods

### *Construction of Metabolic Model*

The metabolic model of *S. stipitis* was constructed following the published protocol (Thiele, I. and B.Ø. Palsson, 2010) and was built based on the genomic and biochemical information of the organism available in its genome project (Jeffries, T.W. and J.R.H. Van Vleet, 2009), KEGG database (Kanehisa, M., et al., 2011) and available biochemical information. An overview of the metabolic model is shown in Figure 1. The metabolites involved in the model have all been verified and balanced with both element and charge based on biochemical information from ChEBI (Degtyarenko, K., et al., 2009) and PubChem compound database (Bolton, E.E., et al., 2008). The model captures cell growth on glucose and xylose. Included in the model are 117 reactions with 66 as reversible reactions and 51 as irreversible reactions (including transport reactions). Fifteen compounds allowed to exchange with external environment are: glucose, xylose, $NH_4^+$, urea, $O_2$, $CO_2$, $SO_4^{2-}$, $H^+$, $HO_4P^{2-}$ ($Pi^{2-}$), $H_2O$, ethanol, acetate, glycerol, xylitol, and biomass. The non-growth associated maintenance requirement was tested within the range of [0.5, 3.5] according to different literatures (Balagurunathan, B., et al., 2012; Caspeta, L., et al., 2012; Guebel, D.V., et al., 1991; Rizzi, M., et al., 1987), and the value of 3.5 mmol ATP/gDCW/h was adopted (DCW stands for Dry Cell Weight). The model constitutes reactions from intermediary metabolism including glycolysis, pentose phosphate pathway, tricarboxylic acid (TCA) cycle, glyoxylate and dicarboxylate metabolism, oxidative phosphorylation, nitrogen metabolism, nicotinate and nicotinamide metabolism. The model also includes reactions of cell mass formation, and synthesis of various precursors and common byproducts such as ethanol, glycerol, xylitol and acetate. Some linear reactions in the model are lumped together for simplification. Transport reactions, including passive diffusion, facilitated diffusion and active transport, are also incorporated. Cell mass reaction in the model was assembled from the macromolecular components of the cells (i.e., proteins, nucleic acids, lipids, and carbohydrates) (Thiele, I., and B.Ø. Palsson, 2010; Senger, R.S., 2010). The contribution of each component to cell mass and the appropriate coefficients for every building block were estimated from the genome data of *S. stipitis* and information of *S. cerevisiae* (Jeffries, T.W., and J.R.H., Van Vleet, 2009; Vanrolleghem, P.A., et al., 1996; Duarte, N.C., et al., 2004). The cell mass term presented here reconnected the cell composition to thirteen precursors along with energy (ATP), redox cofactors ($NAD^+$/NADH and $NADP^+$/NADPH), and nutrients ($Pi^{2-}$, $NH_4^+$ and $SO_4^{2-}$). The thirteen precursors are glutamate, acetyl-CoA, glycerol, oxaloacetate, phosphoenolpyruvate, glucose-6-phosphate, glyceraldehyde-3-phosphate, glutamine, ribose-5-phosphate, pyruvate, erythose-4-phosphate, and 3-Phospho-D-glycerate.

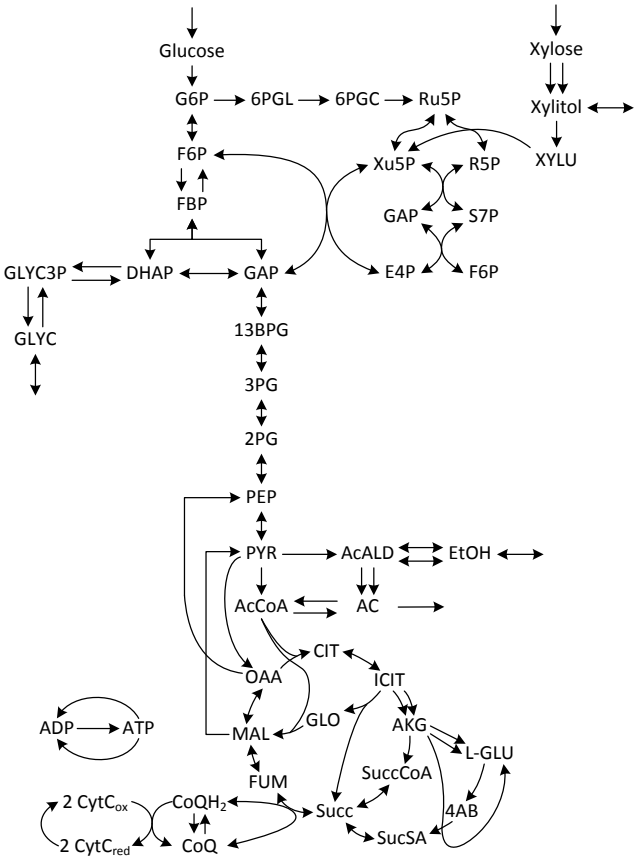


FIGURE 1 OVERVIEW OF THE METABOLIC NETWORK MODEL. The doule arrows in the same direction for a reaction indicate that the enzyme catalyzed the reaction has affinity to different cofactors (NADH/$NAD^+$ and NADPH/$NADP^+$). This also applies to the following figures.

### *Flux Balance Analysis (FBA)*

Flux balance analysis was performed to study the central carbon metabolism of *S. stipitis* using a publicly available COBRA toolbox for Matlab version 2.05 (Schellenberger, J., et al., 2011). The upper limits of uptake rate of xylose and oxygen under various conditions are defined in FBA to predict external secretion rates and internal net fluxes. Other exchange fluxes are constrained accordingly. Maximizing cellular growth rate is used as the objective function

for all FBA simulations. The simulation results were analyzed further to reveal the intracellular mechanism of xylose metabolism.

### *Principal Component Analysis (PCA)*

Principal component analysis (PCA) extracts the directions corresponding to the largest variations among different variables in a high dimensional data set (Jolliffe, L.T., 2002). It has been applied in the metabolomics studies to analyze metabolites profiles at given conditions (Griffin, J.L., 2004). In this work, we apply PCA to analyze the results of a set of specially designed *in silico* experiments to extract the biological sensible information that governs the transient process.

### *Proposed Method: PCA-FBA*

In microbial metabolism, hundreds and even thousands reactions are involved. Although the approaches to study metabolic networks, such as Elementary Mode Analysis (EMA) and Flux Balance Analysis (FBA), could provide detailed flux distributions under different conditions and therefore provide description to different phenotypes, it is very difficult to extract information on the importance of different reactions when a given condition has changed, such as oxygen supply rate, by simple comparison of the flux distributions. In this work, we apply PCA to analyze the results of a series *in silico* experiments to extract such information. In the designed experiments, we incrementally change the factor that we are interested in. The effect of such change will propagate through the whole metabolic network, and show up in various fluxes. If we only compare the two different conditions, such as OTR = 20 and OTR = 0.2, it is very difficult to identify the reactions that play the key role in differentiating these two conditions. However, with the series of incremental changes added between the two conditions, PCA can be applied to extract the key changes caused by the factor, and the loadings of the PCA model provide information on which reactions contribute the most the change.

## Analysis and Validation of S. stipitis Metabolic Network

### *Analysis of the Model*

After the construction of the model, the topological properties and intracellular flux distribution of the central carbon metabolic model were studied using FBA. In all simulations carried out in this work, the model was constrained for cells to grow on minimal defined medium (Jeffries, T.W., et al., 2007). The topological study showed that very few metabolites are highly connected while most metabolites participate only in a few reactions, which indicates that the metabolic network is scale-free. The average reaction participation, i.e. the number of metabolites per reaction, is approximately 4, which indicated that most common reaction mode in the model is totally 4 reactants and products. The topological analysis results are in agreement with the results from other metabolic network models, e.g. *E. coli* (Orth, J.D., et al., 2010) and *S. cerevisiae* (Duarte, N.C., et al., 2004). The reactions that are essential to cell growth were evaluated as whether its removal was fatal to the model. The results indicated that the identified key reactions depend on the carbon source (glucose or xylose) and oxygen condition (aerobic or oxygen-limited). With aerobic glucose culture, it showed that a total of 14 reactions is essential to cell growth. When switched to oxygen-limited condition (glucose), the number changed to 18. For xylose culture, the number is 16 and 26 for aerobic and oxygen-limited respectively. Totally 10 reactions are essential under all conditions. They are reactions in glycolysis, pentose phosphate pathway and urea metabolism. The larger difference of essential reaction number in xylose metabolism under different aeration conditions indicates the higher sensitivity of xylose metabolism to oxygen condition change compared with glucose metabolism, which agrees with experimental findings (Skoog, K., and B. Hahn-Hägerdal, 1990).

### *Validation of the Model*

The predictions of the the growth rate and key products yields with glucose or xylose as carbon source under aerobic or oxygen-limited condition are shown in Figure 2. Under aerobic conditions all the carbon has been used for cell growth and energy generation. No product such as ethanol or acetic acid is produced. Under oxygen-limited condition (0.2 mmol/gDCW/h) the cell growth is inhibited due to limited energy supply and redox balance. Ethanol is the main product. By-products are mainly acetic acid (not shown here) and glycerol or xylitol for glucose or xylose fermentation. These in good agreement with experimental observations (Skoog, K., and B. Hahn-Hägerdal, 1990; Skoog, K., et al., 1992) indicate that the constructed model can capture the metabolic changes caused by different carbon source and oxygen supply. Besides direct predictions of the productions of metabolites from the model, the carbon flux distribution through pentose phosphate pathway was

also studied with glucose or xylose under aerobic or oxygen-limited condition. The carbon distribution through pentose phosphate pathway under aerobic glucose culture is 61.66% which is consistent with the reported value of 57±9% (Fiaux J., et al., 2003). This further confirms the reliability of the model prediction.

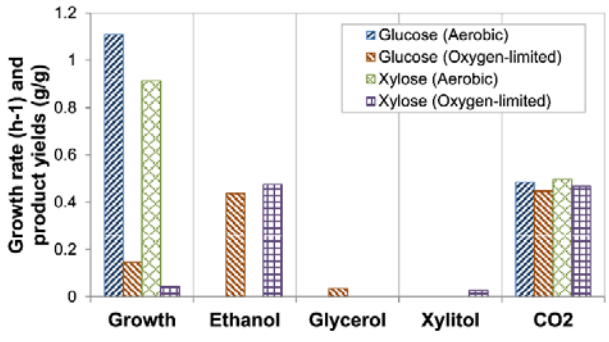


FIGURE 2 GROWTH AND PRODUCTS FORMATION UNDER VARIOUS CARBON SOURCES AND OXYGEN CONDITIONS

## Analysis of Redox Balance in Xylose Metabolism

It is well known that oxygen plays an important role in cell growth, redox balance, functioning of the mitochondria and generation of energy for xylose transport in *S. stipitis* (Skoog, K., and B. Hahn-Hägerdal, 1990). However, how oxygen influences the intracellular flux distribution and redox balance and which reactions would be most important for redox balance are not well understood. In this section we designed a series of *in silico* experiments, and applied PCA to analyze the *in silico* experimental results, with the aim to identify the key reactions that are affected by the introduced change.

### *Phenotype Identification*

#### *1) Designed in Silico Experiments*

In this case study, in order to study how different oxygen availability affects cellular metabolism, we performed FBA to calculate the intracellular fluxes by varying the oxygen supply from 0 to 20 mmol/gDCW/h with a step of 0.001, i.e. totally 20001 runs of experiments. This set of experiments resulted in a 117×2001 matrix, where each column represents the 117 intracellular fluxes under a certain OTR and a xylose uptake rate with upper limit of 10 mmol/gDCW/h. In the model, the flux distribution ratio through NADPH-dependent and NADH-dependent xylose reductase (XR) was set to be 1.0 (the setting and influence of such ratio is discussed later). Phenotype Phase Plane Analysis (PhPP) (Edwards, J.S., et al., 2002; Bell, S., and B. Palsson, 2005) was also carried out under the same conditions for comparison.

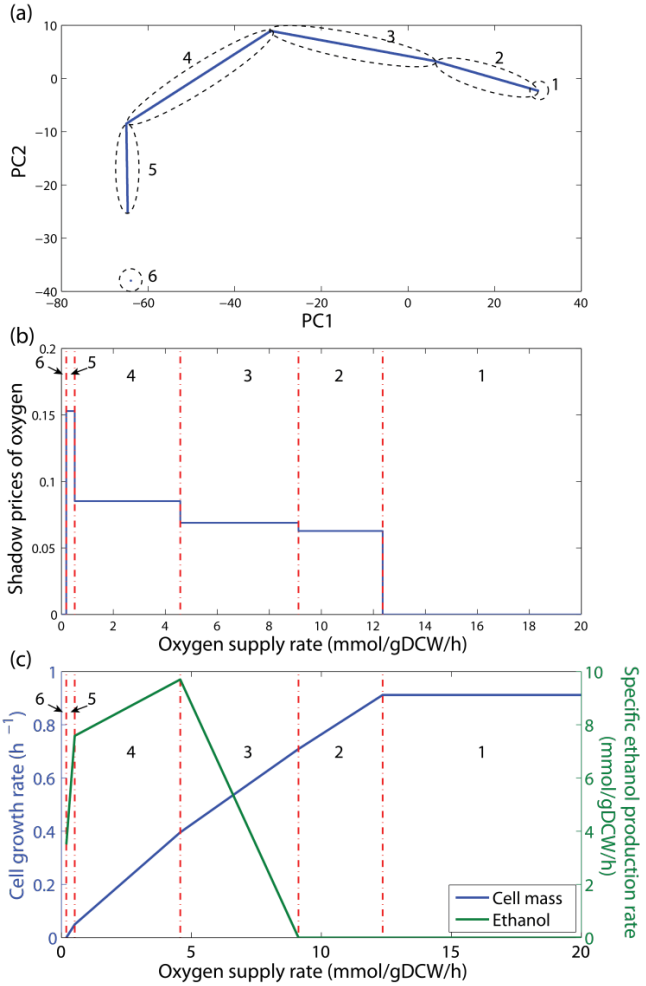


FIGURE 3 PHENOTYPES IDENTIFIED WITH PCA WHEN OXYGEN SUPPLY CHANGES WITHIN [0, 20] mmol/gDCW/h. (a) PHENOTYPES IDENTIFIED BY PCA; (b) PHENOTYPES IDENTIFIED BY PhPP; (c) CELL GROWTH RATES AND SPECIFIC ETHANOL PRODUCTION RATES IN IDENTIFIED PHENOTYPES. The black numbers correspond to the identified phenotypes.

TABLE 1 CHARACTERISTICS SUMMARY OF IDENTIFIED PHENOTYPES

| Pheno-type | Growth limitation | Metabolic product(s) | Main metabolic characteristics |
|---|---|---|---|
| 1 | Xylose | Cell mass | Aerobic growth |
| 2 | Xylose, oxygen | Cell mass, acetic acid | Increasing acetic acid production |
| 3 | Xylose, oxygen | Cell mass, ethanol, acetic acid | Ethanol production and declined acetic acid production |
| 4 | Xylose, oxygen | Cell mass, ethanol, xylitol | Declined ethanol production and increasing xylitol production |
| 5 | Oxygen | Cell mass | Declined ethanol and xylitol production |
| 6 | - | - | Cannot maintain metabolism (no growth) |

#### *2) Phenotypes Identified*

PCA is applied to analyze the *in silico* experimental

results. As shown in Figure 3 (a) where scores corresponding to the first two PCs are plotted, totally six phenotypes of metabolism are identified. The results from PhPP is given in Figure 3 (b), where same number of phenotypes are identified. A closer examination of the PCA results also shows that the same 6 phenotypes are identified. Figure 3(c) plots the cell growth rate and ethanol production rate under different aeration conditions, which reveals some differences among different phenotypes. The main characteristics of the different phenotypes are summarized in TABLE 1.

However, TABLE 1 and Figure 3 (c) do not reveal the cellular details that underlie the specific phenotype. For PhPP, although it can easily identify whether oxygen or carbon source is a limiting factor by examining the shadow price, it is very difficult to extract the cellular details that determinethe change in the shadow price. On the other hand, for the PCA based approach, the limiting factor can be easily identified by checking whether the constraints hit their upper limits. In addition, one significant advantage of the PCA based approach is that it can reveal the cellular details, i.e. the key reactions that differentiate different phenotypes by examing the loading matrix. For example, the key reactions that show dominant changes in phenotype 2 and 3 are plotted in Figure 4 where the metabolic fluxes are colored according to their loadings. From Figure 4, several key differences can be observed. First of all, the importance of TCA cycle for cell growth in phenotype 3 has decreased compared to phenotype 2. Further examination shows that this is caused by turning off of 2-oxoglutarate dehydrogenase due to decreased oxygen supply in phenotype 3, which further leads to an incomplete (or branched) TCA cycle as shown in Figure 5 (also shown as gray TCA cycle in Figure 4 (b) but green TCA cycle in Figure 4 (a)). This branched TCA cycle has been previously reported in *S. cerevisiae* (Vargas, F.A., et al., 2011; Nissen, T.L., et al., 1997). Second, fermentation pathway, i.e. ethanol production, has been activated by the branched TCA cycle to resolve the redox balance of $NADH/NAD^+$ which are indicated by gray in phenotype 2 and red in phenotype 3. Third, due to the decrease of cell growth, the requirement of NADPH has been reduced and caused the down-regulation of fluxes through pentose phosphate pathway shown in Figure 4 by the color of PPP changing from light green in phenotype 2 to light yellow in phenotype 3.

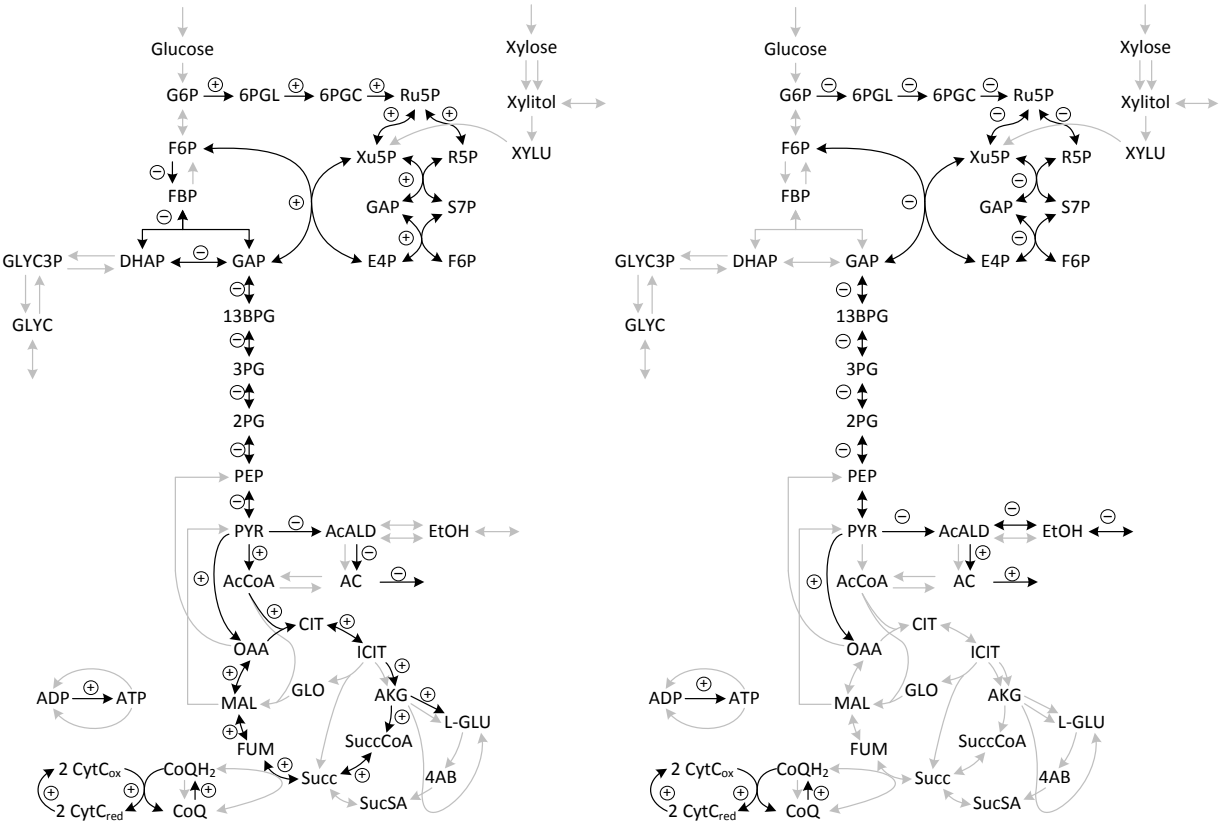


FIGURE 4 METABOLIC MAPS FOR PHENOTYPE 2 (a) AND PHENOTYPE 3 (b) IDENTIFIED IN FIGURE 3.
Minus in circle indicates a negative loading for the reaction while plus in circle indicates positive loading.

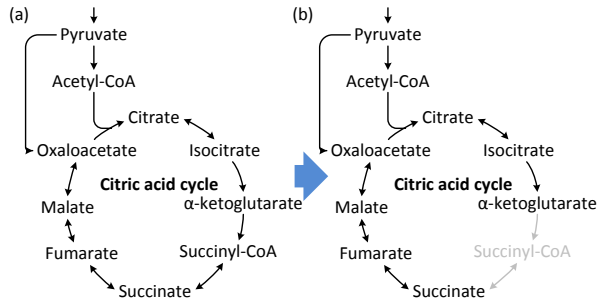


FIGURE 5 TCA CYCLE CHANGE OCCURRED IN PHENOTYPE 3. (a) COMPLETE TCA CYCLE; (b) BRANCHED TCA CYCLE IN PHENOTYPE 3.

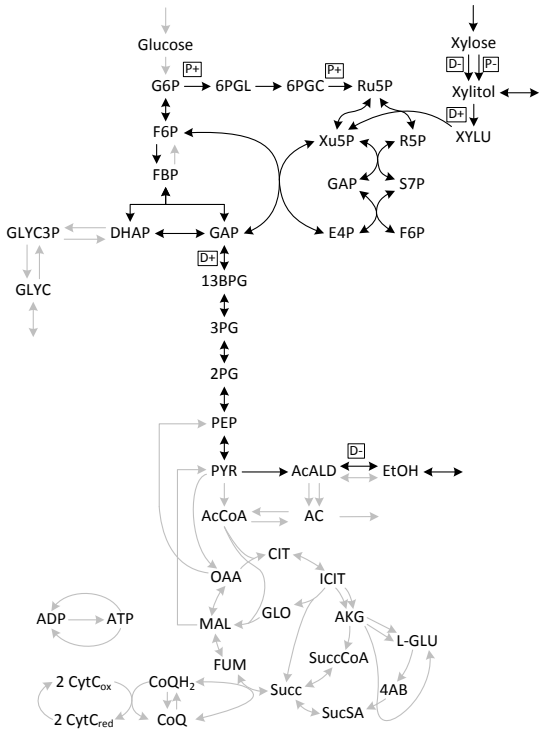


FIGURE 6 METABOLIC MAP FOR PHENOTYPE 5 WITH KEY REACTIONS IDENTIFIED BY PCA COLORED WITH BLACK. The squares with D+, D-, P+ and P- indicate the reactions related to NADH generation, NADH consumption, NADPH generation and NADPH consumption respectively.

### *Identification of Key Reactions in Redox Balance*

In this section, we apply the proposed PCA-FBA method to study the effect of different factors on cell's redox balance. First, we examine the effect of reduced OTR and specially we study the changes that occur in phenotype 5 where OTR range is [0.2, 0.5]. Based on the PCA analysis, the identified key reactions that contribute to redox balance include 4 out of 12 reactions related to NADH/NAD$^+$ (2 related to consumption and 2 related to re-generation of the cofactors) and 3 out of 9 reactions related to NADPH/NADP$^+$ (2 for consumption and 1 for re-generation). The metabolic map with identified key reactions for phenotype 5 is shown in Figure 6. The black reactions shown in Figure 6 are the reactions identified by PCA which are influenced most by OUR change. The identified reactions related with cofactors are marked as well. The importance of the identified reactions in redox balance is shown in TABLE 2. The total consumption rates and generation rates of the identified reactions compensated about 93% of the shifts caused by oxygen changes. It shows clearly that our proposed approach can reveal key information about metabolism shift and therefore help interpret the predictions from metabolic network model and provide insights into microorganism metabolism.

TABLE 2 SHIFT OF COFACTOR CONSUMPTION AND GENERATION IN PHENOTYPE 5

| Cofactor | Fluxes[a] | OUR=0.2 | OUR=0.5 | Shift |
|---|---|---|---|---|
| NADH | (2)(-) | 5.6100 | 12.2400 | 6.6300 |
| | (2)(+) | 6.0079 | 13.1673 | 7.1594 |
| NADPH | (1)(-) | 1.9957 | 4.8527 | 2.8571 |
| | (2)(+) | 2.0041 | 5.1393 | 3.1352 |

[a]Note: the number indicates the number of identified reactions; "-" indicates cofactor being consumed; "+" indicates cofactor being re-generated.

### *Influence of Cofactor Specificity of Xylose Reductase*

It has been reported that *S. stipitis* can ferment xylose to ethanol with little or no xylitol production (Jeffries, T.W., and J.R.H. Van Vleet, 2009), which is due to the dual affinity of XR to both NADH and NADPH instead of mono affinity to NADPH in other yeasts. Therefore, the redox imbalance caused by the NAD$^+$-dependence of xylitol dehydrogenase (XDH) could be reduced (Agbogbo, F.K., et al., 2008) as the black color reactions illustrated in Figure 7. As a result, the flux ratios through NADPH- and NADH-dependent XR would influence the redox balance significantly, especially when oxygen supply was limited.

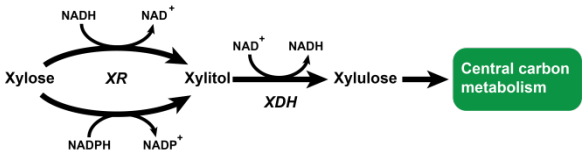


FIGURE 7 ILLUSTRATION OF XYLOSE METABOLISM IN S. STIPITIS.

Many results on experimental study of XR preference to NADPH and NADH have been reported (Hou, X., 2012; Verduyn, C., et al., 1985; Yablochkova, E.N., et al., 2004; Yablochkova, E.N., et al., 2003; Slininger, P.J., et al., 2011). However, the reported results are not consistent with each other (Yablochkova, E.N., et al., 2004; Slininger, P.J., et al., 2011). In addition, all reported enzyme activities are measured *in vitro* with saturated substrate concentration in dilute solution. These all deviate from the *in vivo* condition within the cell. Meanwhile, researchers have tried to apply

protein engineering to alter the cofactor preferences of XR to improve ethanol production and/or to reduce by-products production (Matsushika, A., et al., 2009; Chu, B.C.H., and H. Lee, 2007; Watanabe, S., et al., 2005; Watanabe, S., et al., 2007; Krahulec, S., et al., 2012; Bengtsson, O., et al., 2009; Liang, L., et al., 2007). Therefore, studying the influence of cofactor specificity of XR by altering the flux ratio will help understand the biological details of xylose fermentation in *S. stipitis* and engineered *S. cerevisiae* as well as provide rational design strategy for cofactor engineering.

Here XR activity ratio is defined as the ratio of the flux through the reaction that utilizes NADPH to the flux through the reaction that utilizes NADH when xylose is converted into xylitol. Based on the reported results and general knowledge of the *in vivo* concentrations of NADH/NAD$^+$ and NADPH/NADP$^+$ pools (Bergdahl, B., et al., 2012), in this section we first vary the XR activity ratio within [0, 2] and then its effect on redox balance and ethanol production is investigeted.

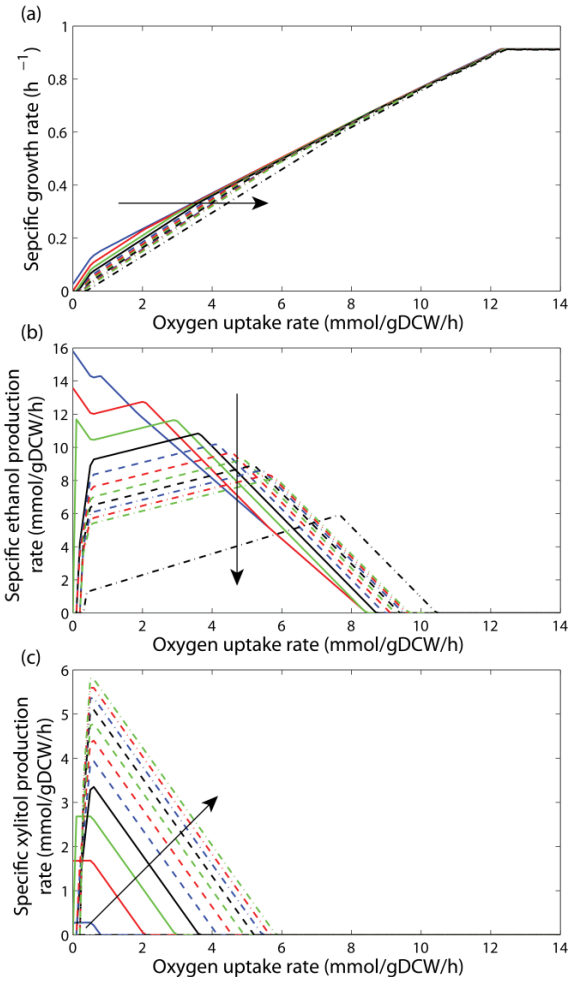


FIGURE 8 INFLUENCES OF FLUX RATIO THROUGH XYLOSE REDUCTASE TO SPECIFIC CELL GROWTH RATE (a), SPECIFIC ETHANOL PRODUCTION RATE (b) AND SPECIFIC XYLITOL PRODUCTION RATE (c). THE BLACK ARROWS IN THE PLOT INDICATE THE INCREASE OF FLUX RATIO.

First we performed simulations to study the general influence of XR activity ratio to model predictions under various oxygenation conditions through FBA. In these experiments, xylose uptake rate is constrained to be 10 mmol/gDCW/h, oxygen supply rate is changed between 0 to 14 mmol/gDCW/h with a step of 0.1, while the XR activity ratio is varied between [0, 2] with a step of 0.2 plus 10 as an extreme case. The resulted cell growth, ethanol production and xylitol production are shown in Figure 8. It shows that the increase of NADH affinity of XR can improve the ethanol production and reduce xylitol production. The results show different patterns in ethanol production rate caused by different ratios through the reactions (shown in Figure 8 (b) as different combination of increase and decrease), which can be used for experimental validation and thus provide insights on flux ratio through different cofactor-linked reactions and intracellular cofactor pool size.

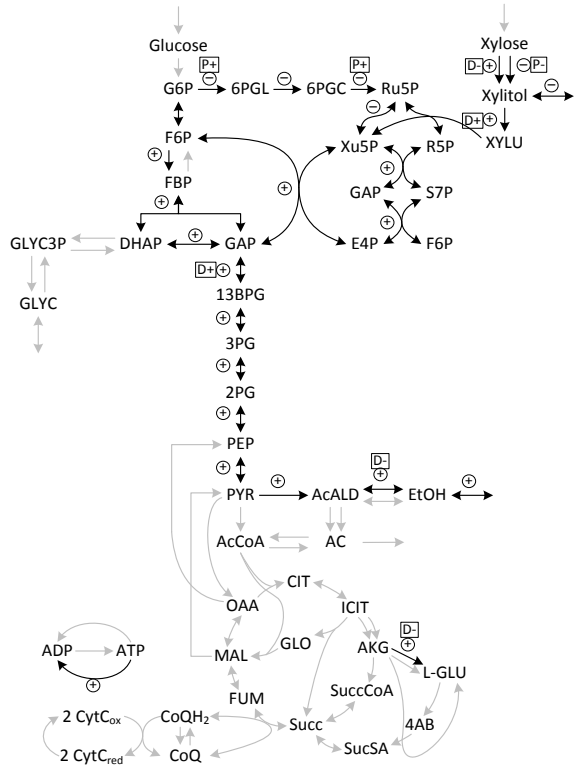


FIGURE 9 METABOLIC NETWORK MAP WITH FLUXES MARKED ACCORDING TO THE LOADINGS OF PCA MODEL FOR ACTIVITY RATIO CHANGE OF XYLOSE REDUCTASE. PLUS IN CIRCLE INDICATES POSITIVE LOADING, MINUS IN CIRCLE INDICATES NEGATIVE LOADING. D+, D-, P+ AND P- IN RECTANGLE CORRESPOND TO NADH GENERATION, NADH CONSUMPTION, NADPH GENERATION AND NADPH CONSUMPTION RESPECTIVELY.

In order to elucidate the cellular details that underlie the predicted cell growth and ethanol production, we carried out a second set of *in silico* experiments, where we fixed both xylose and oxygen uptake rates to 10

mmol/gDCW/h and 0.4 mmol/gDCW/h respectively. The activity ratio of NADPH- and NADH-linked XR is changed incrementally within [0, 2] with a step of 0.001, which results in 2001 *in silico* experiments. PCA is applied to identify the key changes among different reactions when the ratio is changed. The metabolic map marked according to the loadings in PCA model is shown in Figure 9. The PCA result indicates that totally 8 out of 20 reactions involved in cofactor balance are strongly affected by the ratio change, and together they capture 99% of cofactor shifts. Therefore, the identified reactions should receive most attention when cofactor engineering is applied on XR.

## Conclusions

In this work, the central carbon metabolic network of *S. stipitis* was reconstructed. FBA was applied to study the intracellular flux distribution under different oxygen conditions and carbon sources. The predictions of the constructed model agreed well with experimental observations. The topological properties of the metabolic network and the essential reactions under different conditions were identified. The flux distribution through PPP under aerobic glucose culture was consistent with published data. With the model constructed and validated, xylose metabolism by *S. stipitis*, especially redox balance, was studied with application of PCA on intracellular flux profiles. Six phenotypes and the key reactions that contribute to the redox balance under different conditions have been identified. The results showed that PCA is a powerful tool for analyzing output of metabolic model if the *in silico* experiments are designed appropriately. Also, the influence of flux ratios of xylose reductase preference to different cofactors was studied. By integrating PCA with FBA, key cellular details were revealed regarding xylose metabolism. The flux changes caused by cofactor engineering and the identified key reactions for redox balance can be valuable for cofactor engineering strategy.

## ACKNOWLEDGMENT

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